Physiological and Morphological Characterization of Basalt Milkvetch (*Astragalus filipes*): Basis for Plant Improvement

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Abstract

Astragalus filipes Torr. ex A. Gray (basalt milkvetch or threadstalk milkvetch) is a legume that is widely distributed in western North America and holds promise for revegetation and restoration programs in the western United States. Seed of 67 accessions was collected in 2003 from Utah, Nevada, Idaho, Oregon, California, and Washington. Field-collected forage samples from these accessions had nondetectable or low levels of selenium, swainsonine, and nitrotoxins. Accessions were evaluated at Providence and Millville in northern Utah in 2005 and 2006. At Providence accessions from north-central Oregon exhibited comparatively high biomass yield in summer and fall during both years. Basalt milkvetch accessions with low biomass generally had high crude protein concentration. Acid-detergent fiber and neutral-detergent fiber were positively correlated with biomass yield ($r = 0.42$, $P < 0.0001$; $r = 0.57$, $P < 0.0001$, respectively). At Millville accessions from north-central Oregon exhibited comparatively high biomass and seed yield. Seed weight per 100 seeds varied among basalt milkvetch accessions in both years at Millville. Plants at Millville treated with imadicloprid insecticide had greater seed yields than nontreated plants in 2006, but not in 2005. When averaged across sites and years, a high correlation between number of stems and biomass ($r = 0.82$, $P < 0.0001$) indicated that number of stems is a reliable predictor of high biomass and seed yield. Principal component analysis of seven consolidated plant traits identified two principal components that accounted for 60% and 15% of the variation among accessions. The first principal component was negatively correlated with elevation ($r = -0.71$, $P < 0.01$) and positively correlated with latitude ($r = 0.46$, $P < 0.01$). The second principal component was positively correlated with elevation ($r = 0.36$, $P < 0.01$) and negatively correlated with latitude ($r = -0.47$, $P < 0.01$). These results are beneficial in identifying basalt milkvetch accessions that hold promise for plant improvement efforts.

Resumen

La *Astragalus filipes* Torr. ex A. Gray (basalt milkvetch or threadstalk milkvetch) es una leguminosa que está ampliamente distribuida en el oeste de América del Norte y que resulta prometedora para los programas de restablecimiento de vegetación y de restauración en el oeste de los EE.UU. Semillas de 67 accesiones fueron colectadas en el 2003 en Utah, Nevada, Idaho, Oregón, California y Washington. Las muestras de forraje colectadas en el campo de esas accesiones tenían niveles bajos ó no detectables de selenio, trigoldosir y nitrotoxinas. Las accesiones fueron evaluadas en Providencia y Millville en el norte de Utah en el 2005 y el 2006. En Providence, las adhesiones de la región norte central de Oregón exhibieron una alta producción de biomasa comparativa en el verano y el otoño durante ambos años. Las accesiones de "basalt milkvetch" con baja biomasa generalmente tienen una alta concentración de proteína cruda. La fibra de ácido detergente y la fibra neutral detergente fueron correlacionadas positivamente con la producción de biomasa ($r = 0.42$, $P < 0.0001$; $r = 0.57$, $P < 0.0001$, respectivamente). En Millville, las accesiones del norte central de Oregón exhibieron comparativamente una alta producción de biomasa y semillas. El peso de semilla por cada 100 semillas varió entre las adhesiones de "basalt milkvetch" en ambos años en Millville. Las plantas en Millville tratadas con el insecticida imidacloprid tuvieron una mayor producción de semillas que las plantas no tratadas en el 2006, pero no así en el 2005. Cuando promediamos entre los sitios y los años, una alta correlación entre el número de tallos y la biomasa indicó que el número de tallos es un indicador fiable de una alta producción de biomasa y semillas. El análisis del componente principal de siete características consolidadas de plantas identificó dos principales componentes que representan el 60% y el 15% de la variación entre las adhesiones. El primer componente fue correlacionado negativamente con la elevación ($r = -0.71$, $P < 0.01$) y correlacionado positivamente con la latitud ($r = 0.46$, $P < 0.01$). El segundo componente fue correlacionado positivamente con la elevación ($r = 0.36$, $P < 0.01$) y correlacionado negativamente con la latitud ($r = -0.47$, $P < 0.01$). Estos resultados son beneficiosos en identificar las adhesiones de "basalt milkvetch" que resultan prometedoras para los esfuerzos de mejoramiento de la planta.

Key Words: *Astragalus filipes*, restoration, revegetation, sagebrush steppe, western United States

INTRODUCTION

Legumes in rangelands and pastures fix atmospheric nitrogen in association with rhizobia, enhance plant diversity, and increase the quantity and quality of forage for livestock and wildlife (Becker and Crockett 1976; Madison and Robel 2001). Legumes may directly or indirectly increase the productivity of associated species in plant communities by releasing symbiotically fixed nitrogen through root exudates, decaying

This research was partly funded by Great Basin Native Plant Selection and Increase Project through the DOI/BLM Great Basin Restoration Initiative and the USDA Forest Service Rocky Mountain Research Station.

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Manuscript received 15 January 2008; manuscript accepted 28 March 2008.

plant materials, or mycorrhizal hyphae networks (Rumbaugh et al. 1982; Hogh-Jensen and Schjoerring 2000; van der Heijden et al. 2006). Posler et al. (1993) found that five native legumes grown with three different grasses increased grass yield and protein content compared to grasses grown alone. Legumes generally contain more protein and less fiber than grasses at similar stages of maturity (Cherney and Allen 1995).

Basalt milkvetch (*Astragalus filipes* Torr. ex A. Gray), also known as threadstalk milkvetch, is a North American legume native to California, Idaho, Nevada, Oregon, Utah, Washington, northern Mexico, and British Columbia, Canada (Isely 1998). Its high seed production and prominence in recently burned areas makes basalt milkvetch a promising species for restoration and revegetation on western rangelands. Its prevalence after fire may be especially important considering the increasing fire frequency on western rangelands (Whisenant 1990) and the importance of fire as an ecosystem management tool in restoring rangeland function. However, many species of *Astragalus* are toxic to livestock because of problems related to selenium, indolizidine alkaloids such as swainsonine, and/or nitrotoxins (Williams and James 1975; Rumbaugh 1983). Although livestock toxicity problems have not been reported for basalt milkvetch, this species needs to be systematically examined for toxic constituents and other important plant traits before plant improvement efforts proceed.

In the present study, we were interested in determining if broad-based collections of basalt milkvetch differ for toxicity, forage and seed production, forage quality, seed mass, and plant morphological characteristics. We were also interested in knowing if seed production in basalt milkvetch could be increased with the application of imadicloprid insecticide. This information is critical in identifying promising basalt milkvetch accessions for plant improvement programs.

MATERIALS AND METHODS

Seed Collections

Seed of 67 accessions of basalt milkvetch were collected along with associated passport data (latitude, longitude, elevation, site characteristics, and associated species) from sites across six western states of the United States (California, Idaho, Nevada, Oregon, Utah, and Washington) during July and August 2003. Seeds were cleaned and stored in a dark room maintained at 3°C and 20–25% relative humidity until use.

Selenium Analysis

In June and July 2003, six above-ground stems with attached leaves were harvested at a 2-cm stubble height from 10 individual plants at each of the 67 field-collection sites. Harvested plants were placed in paper bags and dried in a greenhouse for 2 mo. One stem from each of the 10 individual plants from each seed collection site was pooled into a composite sample and ground (Cyclotec 1093 Sample Mill, Hoganas, Sweden) to pass through a 1-mm diameter screen. Ground plant samples were analyzed for selenium content at the Utah Veterinary Diagnostic Laboratory, Logan, Utah, by digesting samples in trace mineral grade 5% nitric acid content, which provided a matrix match for the analytical standards. The prepared samples were analyzed by inductively coupled plasma-mass spectrometry (Perkin Elmer, Waltham, MA) and assessed against concentration curves of known standards. Standard curves and quality control samples were analyzed every five samples.

Swainsonine Analysis

Swainsonine content was determined from ground plant samples using the method of Gardner et al. (2001). In brief, a 100-mg dried, ground sample was extracted with chloroform and acetic acid. The resulting solution was placed in an ion-exchange extraction tube and mixed, allowing any swainsonine cations to bind to the resin. To remove the swainsonine from the resin, ammonium hydroxide (1 M, 5 mL) was added, mixed for 15 min, and centrifuged. The aqueous ammonium hydroxide sample solutions were capped and stored at −20°C. Samples and external calibration standards were then analyzed by liquid chromatography-mass spectrometry (Finnigan MAT, San Jose, CA). Swainsonine peak area was quantified based on external calibration standards and then converted to percentage dry weight of the analyzed plant sample.

Nitrotoxin Analysis

The presence or absence of 3-nitropropanol and 3-nitropropionic acid was determined by Fourier transform infrared spectroscopy (FT-IR; Nicolet, Madison, WI; Schoch et al. 1998). In brief, 100 mg of plant sample was weighed into 7-mL screw cap vials, and 4 mL of acetone was added. The samples were capped with Teflon-lined caps, and the samples were mixed by mechanical rotation for 16 hr. Samples were centrifuged, and a 5-μL aliquot was removed with a 10-μL glass syringe for deposition on a KBr microcell for FT-IR analysis. A diffuse reflectance (DRIFTS, Spectra-Tech, Stamford, CT) micro-sample cup was filled with powdered KBr to which 5 μL of the acetone extract was added, and then the sample was allowed to dry for 2 to 3 min under a constant flow of ambient air passing through a standard laboratory fume hood. The sample cup was subsequently placed in the DRIFTS apparatus, and infrared spectra were recorded after 16 scans at $4 \cdot cm^{-1}$ resolution. The presence of 3-nitropropanol (3-NPOH) was determined from absorbance at $1\,552 \cdot cm^{-1}$, and 3-nitropropionic acid was determined from absorbance at $1\,557 \cdot cm^{-1}$.

For each composite sample that tested positive for 3-nitroproponal or 3-nitropropionic acid, each of the 10 individual samples from that particular location was reanalyzed quantitatively to measure the concentration (mg/g NO_2) in the individual samples. For quantitative analysis, 100 mg of each sample was extracted with 2 mL of acetone containing 400 mg/mL potassiun thiocyanate (KSCN) for 16 h. The acetone extract was again analyzed by DRIFTS, and the peak area ratio was measured between $1\,552 \cdot cm^{-1}$ (3-NPOH) and $2\,060 \cdot cm^{-1}$ (KSCN), which was referenced to a calibration curve of known concentration.

Common Garden Studies

During January 2004, the 67 seed accessions of basalt milkvetch (Table 1) were germinated on moistened blotter paper and transplanted to cone-shaped plastic containers (4-cm diameter, 22-cm length) filled with a 3:1 sand:peat moss mixture. The seedlings were grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory at Logan, Utah (lat 41°45′N, long 110°48′W, 1 350 m above sea level) under a day/night temperature regime of about 30/15°C and were watered and fertilized to maintain growth. After one week of growth, about 5 mm of homogenized sieved soil from the 67 collection sites was added to each seedling container for rhizobial inoculation. In addition, a slurry of common rhizobial inoculum (*Astragalus* Special No. 1, EMD Crop Bioscience, Milwaukee, WI) was added to each container after about 3 wk of growth.

After about 90 days of greenhouse growth, seedlings were transplanted to field plots at Providence (lat 41°41′N, long 111°49′W, 1 350 m above sea level) and Millville (lat 41°39′N, long 111°48′W, 1 370 m above sea level) located about 2 km and 9 km south of Logan, Utah, respectively. Soil at Providence is a Nibley silty clay loam (fine, mixed, mesic Aquic Argiustolls), whereas soil at Millville is a Ricks gravelly loam (coarse-loamy over sandy or sand-skeletal, mixed, superactive mesic, Calcic Haploxerolls). The experimental design at Providence was a randomized complete block design with six replications. The experimental design at Millville was a split-plot design with an insecticide treatment (described later) applied on three of the six replications as a whole-plot factor with accession as the subplot factor. At both locations plots consisted of five plants of an accession with a 0.5-m spacing within and between rows. Plots were irrigated only during the establishment year after transplanting, except for plants at Millville, which were also irrigated after insecticide application in 2005 and 2006.

Plant height, number of stems, number of inflorescences, winter mortality, and plant vigor score (on a scale of 0 to 9) were determined for each plot at both sites in both years during late May to early June. Dry-matter yield was determined for plants from Providence. Plants at Providence were harvested at a 5-cm stubble height at about 50% bloom (Miller 1984) during 24–27 June 2005 and on 7 June 2006. Regrowth biomass was also harvested at Providence on 17 October 2005 and 18 October in 2006. At Millville biomass was harvested on 2 August 2005 and 8 August 2006 (after mature seed was harvested), and regrowth biomass was harvested on 26 October 2005. Negligible regrowth occurred at Millville in 2006, so regrowth biomass was not harvested in 2006. Plant samples were oven dried at 60°C for 72 h, and then dry weights were determined.

Forage quality was determined for samples from the June harvest in 2005 and 2006 at Providence. Samples were ground (Clycotec 1093 Sample Mill) to pass through a 1-mm diameter screen. Ground samples were analyzed for crude protein by Utah State University Analytical Laboratories using total combustion procedures (LECO TruSpec C/N analyzer, LECO Corp., St. Joseph, MI). Because of limited funds, a subset of ground samples from the 2006 harvest at Providence was analyzed at the Utah State University Analytical Laboratories for acid-detergent fiber (ADF) following procedures of Association of Official Analytical Chemists (1990) and for neutral-detergent fiber (NDF) according to Goering and Van Soest (1970), as modified by Mertens (1992) at the Utah State Analytical Laboratories.

Seed production was evaluated at Millville only. Insecticide was applied to evaluate the effect of insecticide on seed yield. For three of the six replications, Admire 2 (a flowable insecticide with 21.4% of active ingredient imadicloprid, 1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine; Bayer CropScience, Kansas City, MO) was applied on 2 June 2005 at a rate of 47.1 mL per whole plot. Provado 1.6 (a flowable insecticide with 17.4% imadicloprid) was applied on 8 June 2006 at a rate of 58.8 mL per whole plot on three of the six replications. These rates allowed the application of the same amount of active ingredient in each study year. Irrigation was applied after insecticide application to facilitate plant absorption of the insecticide. Seeds were sequentially harvested from mid-July to early August in both years after pods matured. Seed mass was determined from 100-seed samples.

For principal component analysis, a total of 23 measurement variables (two measurements each for plant height, number of stems, number of inflorescences, plant vigor, June biomass, October biomass, crude protein concentration; one measurement of winter mortality at Providence; two measurements each for August biomass, seed yield, and seed mass; and one measurement each for October biomass and winter mortality at Millville) for each accession were consolidated into seven new variables. This allowed data of similar traits to be combined into a smaller number of variables. For example, a new single biomass variable was consolidated from seven individual biomass harvests using the first principal component score. In this case, the first principal component described 78% of the variation of the seven individual biomass-related measurements. Similarly, the multiple determinations for seed yield, seed weight, crude protein concentration, and plant mortality were consolidated into four new single variables that described 72%, 90%, 71%, and 74% of the total variation of the multiple determinations, respectively. Plant height and vigor score, as well as number of stems and number of inflorescences were consolidated into two new variables that described 82% and 85% of the total variability, respectively. After these seven new variables were synthesized, a second principal component analysis was conducted.

A geographic distance matrix was created following Larson et al. (2004). Morphometric matrices were created by calculating Euclidean distance between each pair of basalt milkvetch accessions for all the measured variables using SAS (Version 9.0; SAS Institute, Cary, NC). Similar matrices were constructed for elevation, mean annual maximum temperature, mean annual minimum temperature, mean annual precipitation, and a combined matrix of the environmental measurements.

Statistical Analysis

For Providence, accession was considered a fixed factor and replication a random factor. For Millville, insecticide treatment (whole plot) and accession (subplot) were considered fixed factors and replication a random factor. Analysis of variance was conducted using Proc. MIXED (SAS, Version 9.0) for both experiments. Because insecticide was not applied prior to the collection of morphological data at Millville, the insecticide effect was not included in the model for morphological traits. Because not all plots produced seeds, there were not sufficient

Table 1. Climatic and geographic information for basalt milkvetch accessions with state, elevation, latitude, longitude, mean annual maximum (Max.) and minimum (Min.) temperatures, and mean annual precipitation (Precip) as well as the first (PC1) and second (PC2) principal component loadings. Means for temperature and precipitation are averages across 30 yr.

					Temperature				
Accession	State	Elevation (m)	Latitude (N)	Longitude (W)	Max. (°C)	Min. (°C)	Precip (mm)	PC1	PC2
Af-3	NV	1 476	41°52′	118°35′	14	0	225	−0.53385	−1.20896
Af-4	WA	519	47°25′	118°41′	16	3	228	−0.93457	−2.52689
Af-5	OR	202	45°35′	119°56′	16	3	258	2.47694	−1.53369
Af-6	WA	653	46°56′	120°15′	18	4	196	−0.50762	−1.59548
Af-7	OR	1 081	44°22′	117°45′	14	0	303	0.32306	−0.62986
Af-8	OR	1 502	42°56′	118°36′	15	0	348	0.47202	0.11212
Af-9	OR	1 396	43°07′	118°16′	16	1	295	−0.33238	−0.48681
Af-10	NV	1 888	38°36′	117°50′	19	1	205	−0.86801	−0.78853
Af-11	CA	1 372	41°28′	120°56′	14	1	575	−0.08427	2.12461
Af-12	OR	1 354	43°39′	118°36′	13	−2	398	−0.26757	0.37222
Af-13	OR	1 315	43°04′	118°46′	15	0	348	0.39124	0.8903
Af-14	OR	1 449	42°48′	118°49′	15	0	348	−0.13001	0.34057
Af-15	CA	1 410	41°36′	120°25′	15	0	399	0.00886	0.57917
Af-16	CA	1 530	41°53′	120°18′	15	0	399	−0.15627	−0.41826
Af-18	OR	1 463	42°28′	119°47′	15	0	399	0.95991	−0.11397
Af-19	OR	1 331	43°26′	119°00′	15	0	348	−0.2482	0.29499
Af-20	OR	1 617	42°56′	119°54′	16	0	201	−0.37104	0.4247
Af-21	OR	1 435	43°10′	120°40′	13	−1	517	−0.18806	−0.4536
Af-22	NV	2 088	41°04′	114°32′	15	−1	294	−0.57277	0.01806
Af-23	OR	1 138	44°10′	120°43′	16	0	278	−0.34503	0.46767
Af-24	OR	1 019	44°09′	120°28′	14	0	363	−0.16667	1.01984
Af-25	OR	1 177	44°03′	120°45′	16	0	278	−0.2312	0.34174
Af-26	OR	699	44°37′	120°20′	13	1	463	0.79079	0.56931
Af-28	OR	611	44°32′	121°16′	14	3	363	−0.8317	−1.61974
Af-29	OR	687	44°51′	120°49′	14	3	363	1.25961	−0.00298
Af-30	OR	443	44°54′	120°27′	13	1	463	2.7939	−0.03192
Af-30.1	OR	625	44°55′	120°31′	14	3	363	1.24175	1.0263
Af-31	OR	424	44°53′	120°26′	13	1	463	2.44987	0.65122
Af-32	OR	873	44°55′	120°15′	13	1	463	0.76653	0.61711
Af-33	OR	920	44°54′	119°42′	13	1	463	1.2669	−0.31237
Af-34	OR	1 162	44°40′	117°37′	14	0	380	−0.42259	−0.66264
Af-36	OR	952	44°26′	119°03′	13	−2	398	1.07086	0.59326
Af-37	OR	1 152	44°44′	119°06′	15	1	456	0.48215	−0.32229
Af-38	ID	1 653	44°14′	112°12′	13	−1	335	−1.39035	0.89813
Af-39	ID	1 628	43°36′	113°20′	7	−4	612	0.10049	−0.79893
Af-41	NV	1 774	41°48′	115°56′	10	−2	741	−0.16655	0.52549
Af-42	OR	1 250	43°15′	117°12′	16	0	367	0.43207	−0.44493
Af-43	ID	1 717	42°17′	115°56′	10	−2	741	−0.8206	1.27504
Af-44	OR	1 446	43°08′	117°28′	14	0	444	0.29959	0.15067
Af-45	ID	1 250	43°26′	116°58′	14	0	444	0.15925	−0.59896
Af-45.1	ID	1 234	43°24′	116°59′	14	0	444	0.52768	0.62894
Af-46	ID	836	44°04′	116°38′	18	3	253	0.03363	1.1213
Af-47	ID	1 700	43°08′	116°43′	14	0	444	0.43618	−0.23343
Af-48	NV	2 347	39°16′	115°25′	17	0	275	−1.83386	−0.09953
Af-49	NV	2 545	39°32′	114°38′	16	0	296	−1.4658	0.75032
Af-50	NV	2 197	39°35′	117°51′	13	0	388	−1.12211	−0.21836
Af-51	NV	2 376	39°36′	117°53′	13	0	388	−1.20427	0.2113
Af-52	NV	2 421	39°19′	116°31′	17	1	213	−1.46704	1.40106
Af-53	NV	2 258	39°19′	117°07′	17	1	213	−0.3722	1.70722
Af-54	NV	2 262	39°15′	117°09′	17	1	213	−0.9122	1.07663
Af-55	WA	692	47°45′	119°21′	15	3	290	−0.87458	−2.94979

Table 1. Continued.

Accession	State	Elevation (m)	Latitude (N)	Longitude (W)	Temperature Max. (°C)	Temperature Min. (°C)	Precip (mm)	PC1	PC2
Af-56	WA	552	47°21′	118°28′	16	2	367	−0.75492	−1.89821
Af-58	OR	1 055	44°24′	120°58′	16	0	278	−0.32755	−1.61933
Af-59	OR	1 317	42°26′	121°11′	15	0	429	−0.26867	−0.12968
Af-60	CA	1 711	40°42′	120°53′	14	1	575	0.29386	0.75788
Af-62	OR	1 172	44°17′	118°57′	13	−2	398	−0.00513	1.62868
Af-63	OR	1 501	42°18′	120°20′	15	0	399	−0.19073	0.02339
Af-64	OR	1 302	42°43′	120°43′	13	−1	517	−0.07894	0.27998
Af-65	NV	2 088	41°04′	114°32′	15	−1	294	−1.16074	−0.22797
Af-66	NV	2 499	41°41′	117°34′	15	1	354	−1.08202	−0.21238
Af-67	ID	2 080	43°24′	113°02′	14	0	265	−0.7466	−1.25157
Af-68	ID	2 201	44°20′	113°31′	11	−5	342	−1.09875	0.61636
Af-69	UT	1 874	41°54′	113°58′	15	0	309	−0.6148	0.93949
Af-70	OR	408	44°54′	120°26′	13	1	463	1.91501	0.15856
Af-75	OR	265	45°37′	119°43′	16	3	258	1.82171	−1.98586
Af-76	OR	479	44°54′	120°24′	13	1	463	2.2889	−0.09205
Af-77	OR	1 595	43°26′	117°55′	16	1	295	0.08747	0.87534

degrees of freedom to test the insecticide effect on seed mass. Data were root-transformed when required to meet normality and homoscedasticity assumptions. Significance was tested at $\alpha = 5\%$, post hoc mean separations were conducted using the Tukey Test, and Pearson correlation coefficients were calculated with SAS using Proc CORREL (SAS, Version 9.0). Principal component analysis was conducted using Proc FACTOR in SAS (Version 9.0). Relationships between morphometric traits and geographic distance, elevation, mean annual maximum temperature, mean annual minimum temperature, and precipitation were examined with the Mantel test statistic (Mantel 1967) using the MXCOMP procedure of NTSYSpc (Rohlf 1998).

RESULTS

Selenium, Swainsonine, and Nitrotoxin Content

Only Af-68 tested positive for swainsonine, and only 2 of the 10 individual samples of this accession contained detectable levels (Table 2). Similarly, only nine accessions contained detectable levels of nitrotoxins (3-nitro-1-propanol), and all concentrations were low (0.03–0.48 mg/g NO_2). Only seven accessions had detectable selenium concentrations that ranged from 1.04 to 1.77 μg/g. All other accessions contained selenium concentrations below 1.00 μg/g.

Forage and Seed Production and Forage Quality

Accessions varied significantly for June and October biomass at Providence in both 2005 and 2006 (Table 3). In 2005 June biomass ranged from 2 to 44 g · plot^{-1}, and October biomass ranged from 1 to 69 g · plot^{-1} (Table 4). In 2006 June biomass ranged from 2 to 114 g · plot^{-1}, and October biomass ranged from 1 to 59 g · plot^{-1}. At Millville, August and October 2005 biomass varied significantly and ranged from 4 to 98 g · plot^{-1} for August and 0 to 17 g · plot^{-1} for October. The short (50-d) time period between the August and October harvests at Millville resulted in negligible growth in October for some accessions. In 2006 August biomass differed significantly among accessions, ranging from 5 to 147 g · plot^{-1}. There was no harvestable biomass in October 2006 at Millville. No significant insecticide effect was observed for biomass yield at Millville. Accessions exhibited positive correlations for biomass production between Providence and Millville (in 2005, $r = 0.52$, $P < 0.01$; in 2006, $r = 0.32$, $P < 0.01$). Accessions collected from north-central Oregon had high biomass, whereas accessions from Nevada, Washington, Utah, and some from Oregon and Idaho consistently produced relatively low biomass across all harvests.

Table 2. Concentration of detected natural toxins in basalt milkvetch accessions.

Accession[1]	Swainsonine (mg · g^{-1})	Nitrotoxins[2] (mg · g^{-1})	Selenium (μg · g^{-1})
Af-19	—[3]	—	1.04
Af-22	—	0.08 ± 0.14	—
Af-25	—	—	1.34
Af-30.1	—	—	1.23
Af-39	—	0.21 ± 0.22	—
Af-43	—	0.14 ± 0.12	—
Af-44	—	0.10 ± 0.10	—
Af-45	—	0.48 ± 0.57	1.04
Af-46	—	0.18 ± 0.12	1.77
Af-47	—	0.21 ± 0.32	—
Af-49	—	0.25 ± 0.34	—
Af-50	—	0.03 ± 0.04	—
Af-52	—	—	1.10
Af-68	0.10[4]	—	1.05

[1]Basalt milkvetch accessions not listed in this table had no detectable concentrations of swainsonine and nitrotoxins, and selenium concentrations below 1.0 μg · g^{-1} in the composite plant samples.

[2]Ten individual samples were analyzed quantitatively for each basalt milkvetch accession that tested positive in the initial nitrotoxin screening.

[3]Dashed lines indicate no detectable concentrations.

[4]All 10 individual plant samples were analyzed from Af-68 for swainsonine, but only two samples had detectable concentrations of swainsonine (0.66 and 0.03 mg · g^{-1}).

Table 3. Analyses of variance of basalt milkvetch accessions for June biomass, August biomass, October biomass, plant height, number of stems, number of inflorescences, plant vigor, crude protein concentration (CPC), seed mass, and seed yield in 2005 and 2006, acid detergent fiber (ADF) and neutral detergent fiber (NDF) in 2006, and the contrast between years (2005 and 2006) at Providence and Millville.

Factor	2005		2006		2005 vs. 2006	
	df	F^1	df	F^1	df	F^1
Providence						
June biomass, $g \cdot plot^{-1}$	66, 328	7.62**	66, 326	5.04**	1, 4.9	190.84**
October biomass, $g \cdot plot^{-1}$	66, 328	7.68**	66, 332	6.5**	1, 5	122.89**
Plant height, cm	66, 328	5.33**	66, 328	2.58**	1, 5	0.46^{ns}
No. of stems	66, 325	8.35**	66, 327	8.32**	1, 5	336.61**
No. of inflorescences	66, 328	3.94**	66, 329	3.98**	1, 5	60.7**
Plant vigor score	66, 327	5.59**	66, 327	3.02**	1, 5	54.76**
CPC, $g \cdot kg^{-1}$ dry matter	66, 328	3.69	66, 323	4.42	1, 5	0.68^{ns}
ADF, $g \cdot kg^{-1}$ dry matter	—	—	16, 48	4.47	—	—
NDF, $g \cdot kg^{-1}$ dry matter	—	—	16, 46	4.22	—	—
Millville						
August biomass, $g \cdot plot^{-1}$	66, 316	11.89**	66, 316	10.77**	1, 5	243.63**
October biomass, $g \cdot plot^{-1}$	66, 322	12.9**	—	—	—	—
Plant height, cm	66, 322	4.12**	66, 313	3.92**	1, 5.1	0.04^{ns}
No. of stems	66, 323	7.65**	66, 314	9.47**	1, 5	1189.4**
No. of inflorescences	66, 325	3.55**	66, 316	4.95**	1, 5.1	517.5**
Plant vigor score	66, 324	3.87**	66, 312	5.41**	1, 4.9	594.2**
Seed mass, $g \cdot 100\ seeds^{-1}$	66, 194	4.33**	66, 251	6.97**	1, 5.1	44.24**
Seed yield, g						
Accession (A)	66, 260	3.30**	66, 258	3.44**	1, 5.1	31.78**
Insecticide treatment (T)	1, 260	0.27^{ns}	1, 258	23.42**	—	—
A × T	66, 260	1.12^{ns}	66, 258	1.04^{ns}	—	—

[1]ns = not significant, * = $P < 0.05$, ** = $P < 0.01$.

Table 4. Mean, standard deviation, and range of basalt milkvetch accessions for June biomass, August biomass, October biomass, plant height, number of stems, number of inflorescences, plant vigor score, crude protein concentration (CPC), acid detergent fiber (ADF), neutral detergent fiber (NDF), seed yield, and seed mass in 2005 and 2006.

Measurement	2005			2006		
	Mean	SD	Range	Mean	SD	Range
Providence						
June biomass, $g \cdot plot^{-1}$	13	9	2–44	38	24	2–114
October biomass, $g \cdot plot^{-1}$	7	9	1–69	14	11	1–59
Plant height, cm	26	5	16–37	26	4	16–34
No. of stems	4	2	2–10	14	7	4–32
No. of inflorescences	4	3	0–17	19	15	1–82
Plant vigor score	5	1	3–7	4	1	2–5
CPC, $g \cdot kg^{-1}$ dry matter	140	17	95–179	147	15	118–202
NDF, $g \cdot kg^{-1}$ dry matter	—	—	—	420	31	361–461
ADF, $g \cdot kg^{-1}$ dry matter	—	—	—	348	24	308–380
Millville						
August biomass, $g \cdot plot^{-1}$	32	22	4–98	58	34	5–147
October biomass, $g \cdot plot^{-1}$	3	4	0–17	—	—	—
Plant height, cm	26	4	19–37	29	5	18–36
No. of stems	5	2	2–13	21	10	6–37
No. of inflorescences	4	4	0–30	41	23	2–79
Plant vigor score	5	1	3–7	4	1	2–7
Seed yield, $g \cdot plot^{-1}$	1	2	0–10	3	2	0–5
Seed mass, $g \cdot 100\ seeds^{-1}$	0.4	0.1	0.2–0.8	0.5	0.1	0.2–0.7

Accessions differed significantly for crude protein concentration in both 2005 and 2006 (Table 3). Crude protein concentration ranged from 95 to 179 $g \cdot kg^{-1}$ (mean = 140 $g \cdot kg^{-1}$) in 2005 and from 118 to 202 $g \cdot kg^{-1}$ (mean = 147 $g \cdot kg^{-1}$) in 2006. A relatively small (but significant) negative correlation ($r = -0.23$, $P < 0.01$) was observed between biomass production and crude protein concentration in 2005. The NDF of accessions varied significantly from 361 to 461 $g \cdot kg^{-1}$ (mean = 420 $g \cdot kg^{-1}$), and ADF ranged from 308 to 380 $g \cdot kg^{-1}$ (mean = 348 $g \cdot kg^{-1}$). Positive correlations were observed between biomass and ADF ($r = 0.42$, $P < 0.0001$) and between biomass and NDF ($r = 0.57$, $P < 0.0001$).

Accessions differed significantly for seed yield in 2005 and 2006 at Millville. The insecticide effect was not significant in 2005, but was significant in 2006 (Table 3). The interaction between insecticide and accessions was not significant in either 2005 or 2006. The range of seed yield was 0 to 10 $g \cdot plot^{-1}$ in 2005 (Table 4). The accession and insecticide main effects were significant for seed yield in 2006 (Table 3), and seed yields ranged from 0 to 5 $g \cdot plot^{-1}$ (Table 4). In 2006 the insecticide treatment resulted in 62% higher seed yield than the treatment with no insecticide. Generally, accessions produced 163% greater seed yield in 2006 than in 2005 (Tables 3 and 4).

Accessions differed significantly for seed mass in both 2005 and 2006 (Table 3) with a range from 0.2 to 0.8 $g \cdot 100\ seeds^{-1}$ (Table 4). Seed mass was more closely correlated with biomass in 2005 ($r = 0.49$, $P < 0.0001$) and 2006 ($r = 0.60$, $P < 0.0001$) than with seed yield in 2005 ($r = 0.30$, $P < 0.05$) or in 2006 ($r = 0.51$, $P < 0.05$; data not shown).

Plant Mortality and Morphological Measurements

Considerable variation was detected in mortality over winter at both study sites. At Providence winter mortality in 2005 ranged from 0% to 30% with a mean of 9%. In 2006 cumulative winter mortality ranged from 0% to 73% with a mean of 23%. At Millville in 2005, winter mortality ranged from 7% to 53% with a mean of 25%, and in 2006 cumulative winter mortality ranged from 13% to 83% with a mean of 35%.

Significant variation for plant height, number of stems per plant, number of inflorescences per plant, and plant vigor score was detected among basalt milkvetch accessions (Table 3). Plant height varied significantly at Providence in 2005 (range of 16 to 37 cm) and 2006 (range of 16 to 34 cm) and at Millville in 2005 (range of 19 to 37 cm) and 2006 (range of 18 to 36 cm; Table 4). Number of stems differed significantly among accessions at Providence in 2005 (range of 2 to 10) and 2006 (range of 4 to 32), and at Millville in 2005 (range of 2 to 13) and 2006 (range of 6 to 37). In addition, number of inflorescences differed significantly among accessions at Providence in 2005 (range of 0 to 17) and 2006 (range of 1 to 82), and at Millville in 2005 (range of 0 to 30) and 2006 (range of 2 to 79). Accessions also differed significantly for plant vigor score at Providence in 2005 (range of 3 to 7) and 2006 (range of 2 to 5), and at Millville in 2005 (range of 3 to 7) and 2006 (range of 2 to 7). Significant differences were observed between 2005 and 2006 for number of stems, number of inflorescences, and plant vigor at both field sites, but not for plant height (Table 3). Number of stems and number of inflorescences were significantly greater in 2006 (250% and 375%, respectively), but plant vigor score decreased (20%) from 2005 to 2006.

Table 5. Pearson correlation coefficients (r) and their associated significance levels across basalt milkvetch accessions for Providence in 2005 and 2006. Variables include plant height (Ht), number of stems (St), number of inflorescences (If), plant vigor scores (Vs), June biomass (Jb), and October biomass (Ob).

	Ht	St	If	Vs
2005				
Ht	—	—	—	—
St	0.474**	—	—	—
If	0.510**	0.837**	—	—
Vs	0.907**	0.618**	0.559**	—
Jb	0.559**	0.865**	0.859**	0.602**
Ob	0.361**	0.52**	0.528**	0.378**
2006				
Ht	—	—	—	—
St	0.609**	—	—	—
If	0.607**	0.775**	—	—
Vs	0.880**	0.751**	0.660**	—
Jb	0.651**	0.859**	0.803**	0.769**
Ob	0.521**	0.709**	0.676**	0.636**

** = $P < 0.01$.

At Providence, the Pearson correlation coefficient between number of stems and June biomass was significant in 2005 ($r = 0.87$, $P < 0.0001$) and 2006 ($r = 0.86$, $P < 0.0001$; Table 5). Correlations between plant height and June biomass were significant in 2005 ($r = 0.56$, $P < 0.0001$) and 2006 ($r = 0.65$, $P < 0.0001$). Also, October biomass was significantly correlated with number of stems in 2005 ($r = 0.52$, $P < 0.0001$) and 2006 ($r = 0.71$, $P < 0.0001$). Similarly, October biomass was correlated with plant height in 2005 ($r = 0.36$, $P < 0.0001$) and 2006 ($r = 0.52$, $P < 0.0001$). Number of inflorescences at Providence was highly correlated with June biomass in 2005 ($r = 0.86$, $P < 0.0001$) and 2006 ($r = 0.80$, $P < 0.0001$). At Providence plant vigor was more strongly correlated with plant height in 2005 ($r = 0.91$, $P < 0.0001$) and 2006 ($r = 0.88$, $P < 0.0001$) than with any other plant characteristic.

At Millville August biomass was more strongly correlated with number of stems in 2005 ($r = 0.68$, $P < 0.0001$) and 2006 ($r = 0.73$, $P < 0.0001$) than with plant height in 2005 ($r = 0.44$, $P < 0.0001$) and 2006 ($r = 0.54$, $P < 0.0001$), number of inflorescences in 2005 ($r = 0.57$, $P < 0.0001$) and 2006 ($r = 56$, $P < 0.0001$), and plant vigor in 2005 ($r = 0.50$, $P < 0.0001$) and 2006 ($r = 0.45$, $P < 0.0001$; Table 6). Seed yield was highly correlated with number of inflorescences in 2005 ($r = 0.88$, $P < 0.0001$) and 2006 (r = 0.53, $P < 0.0001$) and August biomass in 2005 ($r = 0.75$, $P < 0.0001$) and 2006 ($r = 0.71$, $P < 0.0001$). Number of stems was significantly correlated with seed yield in 2005 ($r = 0.67$, $P < 0.0001$) and 2006 ($r = 0.46$, $P < 0.0001$), and these correlations were higher than those of plant height and plant vigor with seed yield.

Principal Component Analysis

The first principal component (PC1) described 60.5% of the total variation among accessions, whereas the second principal

Table 6. Pearson correlation coefficients (r) and their associated significance levels across basalt milkvetch accessions for Millville in 2005 and 2006. Variables include plant height (Ht), number of stems (St), number of inflorescences (If), plant vigor scores (Vs), August biomass (Ab), October biomass (Ob), and seed yield (Sy).

	Ht	St	If	Vs	Ab	Ob
2005						
Ht	—	—	—	—	—	—
St	0.411**	—	—	—	—	—
If	0.438**	0.742**	—	—	—	—
Vs	0.785**	0.595**	0.555**	—	—	—
Ab	0.439**	0.677**	0.572**	0.496**	—	—
Ob	0.131**	0.419**	0.358**	0.183**	0.557**	—
Sy	0.327**	0.67**	0.876**	0.45**	0.751**	0.317**
2006						
Ht	—	—	—	—	—	—
St	0.558**	—	—	—	—	—
If	0.634**	0.772**	—	—	—	—
Vs	0.465**	0.554**	0.488**	—	—	—
Ab	0.542**	0.732**	0.562**	0.451**	—	—
Ob	—	—	—	—	—	—
Sy	0.359**	0.461**	0.525**	0.262**	0.714**	—

** = $P < 0.01$.

component (PC2) described an additional 15.4% of the total variation. The PC1 loadings were high for biomass (0.94), seed yield (0.87), combined plant height and vigor score (0.80), and combined number of stems and inflorescences (0.93), and low for crude protein (−0.67) and overwinter mortality (−0.63; Table 7). The PC2 loadings were high for seed mass (0.71).

A strong negative correlation was observed between PC1 and elevation of the collection sites ($r = -0.71$, $P < 0.0001$; Table 8). Accessions collected from lower elevations tended to have greater biomass, seed yield, plant height, number of stems and inflorescences, and plant vigor score, but lower crude protein concentration and winter mortality than those from higher elevations. Accessions from Washington (Af-4, Af-6, Af-55, and Af-56) and one accession from Oregon (Af-28) were considered outliers (Fig. 1b). Although these accessions were collected from low elevations, their PC1 coefficients were lower than those of other low-elevation accessions. Accessions from Nevada, Washington, and California generally clustered separately. However, Oregon and Idaho accessions exhibited a wide dispersion on the PC1 axis.

PC2 was positively correlated with elevation ($r = 0.36$, $P = 0.003$). The significant positive correlation between PC2 and elevation indicated that accessions from higher elevations tended to produce seeds with greater seed mass than those from lower elevations. PC2 separated Washington accessions from those from Nevada, Idaho, and California (Fig. 2). Principal component analysis showed that accessions from north-central Oregon generally had greater biomass and seed yield, number of stems and inflorescences, and greater plant height than other accessions. A scatter plot of PC1 and PC2 revealed that basalt milkvetch accessions generally clustered by state for Nevada, Washington, and California, but not for Idaho and Oregon (Fig. 1a).

A positive correlation ($r = 0.46$, $P < 0.0001$) was detected between latitude and PC1, but a negative correlation was observed between longitude and PC1 ($r = -0.49$, $P < 0.0001$; Table 8). To evaluate the redundancy among the three geographic variables (elevation, latitude, and longitude), a type III sums of squares analysis was employed. This analysis for PC1 indicated that 1) elevation was significant even after

Table 7. First (PC1) and second (PC2) principal component loadings for each consolidated trait across basalt milkvetch accessions. HVS is the consolidated trait from plant height and plant vigor score, whereas SIN is the consolidated trait for number of stems and number of inflorescences.

Combined traits	PC1 loadings	PC2 loadings
Biomass	0.94296	−0.00719
Seed yield	0.86839	0.0956
Seed mass	0.5002	0.71312
HVS	0.79667	−0.35545
SIN	0.93034	−0.12901
Crude protein	−0.66877	0.46767
Mortality	−0.62771	−0.45086

Table 8. Pearson's correlation coefficients for the first (PC1) and second (PC2) principal components with elevation, longitude, latitude, mean annual maximum temperature (Max.), mean annual minimum temperature (Min.), and precipitation (Precip) across basalt milkvetch accessions.

	PC1[1]	PC2[1]
Elevation	−0.71**	0.36**
Longitude	−0.49**	0.11ns
Latitude	0.46**	−0.47**
Max.	−0.19ns	−0.14ns
Min.	0.25*	−0.35**
Precip	0.28*	0.26*

[1]ns = not significant, * = $P < 0.05$, ** = $P < 0.01$.

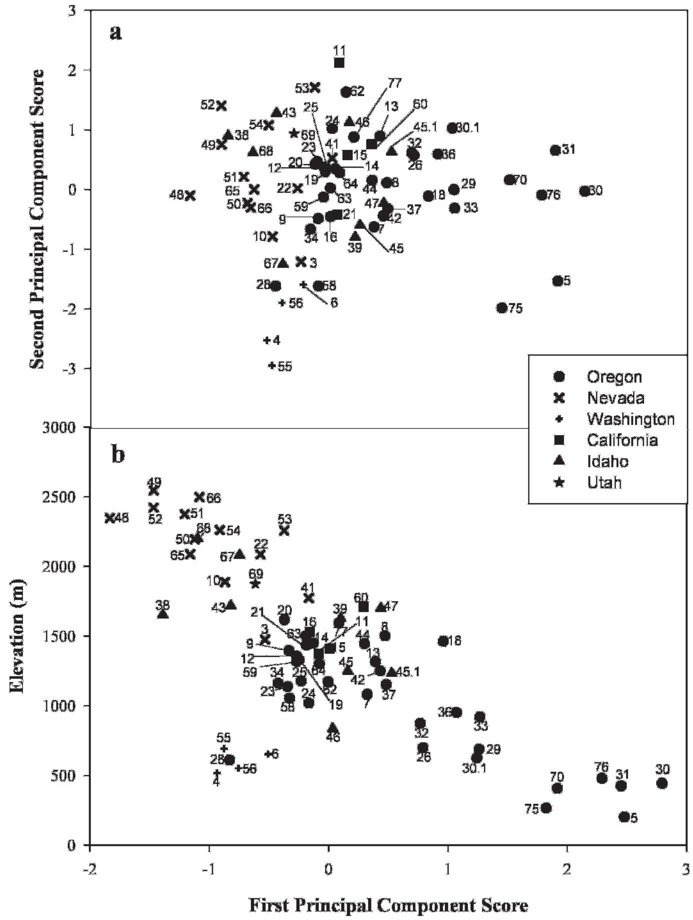

Figure 1. Scatter plots of the first principal component score with **a**, the second principal component, and **b**, elevation of 67 basalt milkvetch accessions.

latitude and elevation were fit in the model and 2) latitude was significant even after longitude and elevation were fit in the model. However, longitude was not significant when elevation and latitude were previously fit in the model. A negative correlation was observed between PC2 and latitude ($r = -0.47$, $P < 0.0001$), and no correlation was observed between PC2 and longitude ($r = 0.11$, $P = 0.38$). Type III sums of squares analysis for PC2 indicated that 1) elevation was significant even after latitude and elevation were fit in the model and 2) longitude was significant even after latitude and elevation were fit in the model. Latitude, however, was not significant when elevation and latitude were previously fit in the model. Further, the Pearson's correlation coefficient was negative between latitude and elevation ($r = -0.84$, $P < 0.0001$) and was positive between longitude and elevation ($r = 0.62$, $P < 0.0001$).

PC1 was positively correlated with precipitation ($r = 0.28$, $P = 0.02$) and mean annual minimum temperature ($r = 0.25$, $P = 0.04$), but not with mean annual maximum temperature ($r = -0.19$, $P = 0.11$). PC2 was positively correlated with mean annual precipitation ($r = 0.26$, $P = 0.04$), negatively correlated with mean annual minimum temperature ($r = -0.35$, $P = 0.003$), and not correlated with mean annual maximum temperature ($r = -0.14$, $P = 0.24$; Table 8). A significant positive correlation ($r = 0.34$, $P = 0.002$) was observed be-

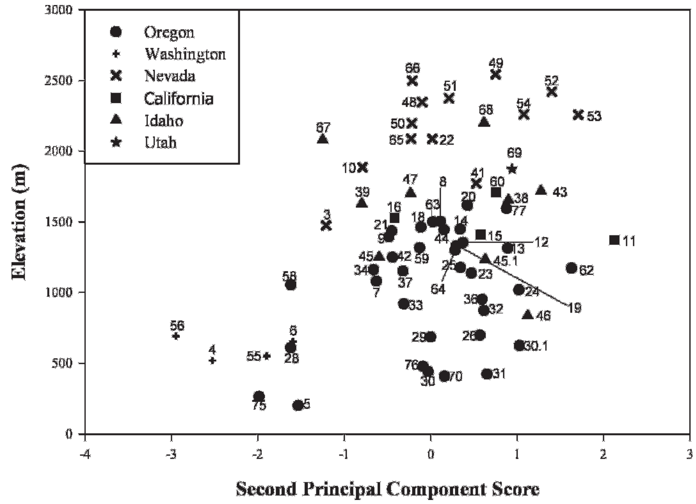

Figure 2. Relationship between elevation and the second principal component score of 67 basalt milkvetch accessions.

tween geographic distance and morphometric distance. Morphometric distance was not significantly correlated with mean annual maximum temperature ($r = 0.07$, $P = 0.18$) or mean annual precipitation ($r = 0.06$, $P = 0.18$), but was positively correlated with elevation ($r = 0.58$, $P = 0.002$) and mean annual minimum temperature ($r = 0.20$, $P = 0.016$).

DISCUSSION

Many species of *Astragalus* and *Oxytropis* are poisonous and cause considerable economic losses to the livestock industry (Kingsbury 1964; James and Nielsen 1988). Three main toxins are of concern, including swainsonine, nitrotoxins (3-NPOH or 3-nitropropionic acid), and selenium. Swainsonine (1,2,8-trihydroxyocta-hydroindolizidine) is an alkaloid alpha-mannosidase inhibitor that causes locoism, a neurological disease (James and Panter 1989). Swainsonine was detected in only one accession (Af-68), which was above the toxic threshold level for swainsonine (< 0.01 mg $\cdot$ g^{-1}; Ralphs et al. 2008). Most basalt milkvetch accessions did not contain detectable levels of nitrotoxins. However, nine accessions had low (but detectable) amounts of 3-nitropropanol, which may be potentially toxic to livestock (Williams 1981). Selenium-containing soils are widespread in the Intermountain Region of the western United States, and selenium-accumulators growing on these soils can cause acute or chronic toxicity in livestock. Typically, plants with selenium contents below 2.00 µg $\cdot$ g^{-1} are not a concern for grazing animals if consumed only as a small proportion of an animal's diet (Kip Panter, personal communication, May 2008). No basalt milkvetch accessions reached that threshold level.

Number of stems was closely related to biomass yield, which was closely correlated with seed yield. These correlations suggest that selecting plants with many stems would generally result in plants with increased biomass and seed yield in basalt milkvetch. As a result, numbers of stems would be a rapid, reliable predictor of accessions with high biomass and seed yield.

Some basalt milkvetch accessions exhibited considerable plant mortality between the October harvest and the following spring. In our study Af-31 exhibited no winter mortality at Providence and only 13% mortality at Millville across two winters, despite originating at a 424-m elevation. Accessions generally exhibited greater mortality at Millville (35%) than at Providence (23%) across two winters, perhaps resulting from a shorter time interval between the harvests at Millville (about 2 mo) compared to Providence (4 mo). A late harvest date was reported as a cause of significant winter mortality in Illinois bundleflower (*Desmanthus illinoensis* [Michx.] MacMill. ex B. L. Rob & Fernald; DeHaan et al. 2003).

Many variables such as temperature, drought, nutrient availability, growth stage, plant genetics, and herbivory influence forage quality (Buxton and Mertens 1995). In our study differences in forage quality among basalt milkvetch accessions could be due to differences in plant maturity and plant genetics because field plots were harvested when the entire plot was at about 50% bloom (Miller 1984). As a result, not all accessions were at the same exact stage of maturity when harvested. Our results showed that forage quality was inversely correlated with biomass yield, similar to results with other species (White and Wight 1984; van der Wal et al. 2000).

We observed greater crude protein concentration in 2006 than in 2005 (Table 3), perhaps due to a greater effectiveness at scavenging nitrogen with a more expansive root system or a more effective legume-*Rhizobium* symbiosis in 2006. When averaged across both years, crude protein concentration was 144 $g \cdot kg^{-1}$ dry matter (DM), which is higher than 13 of 15 legumes native to the north-central United States (mean = 128 $g \cdot kg^{-1}$ DM; McGraw et al. 2004). In our study ADF and NDF varied among basalt milkvetch accessions. High ADF indicates low digestibility (Van Soest et al. 1991), and high NDF indicates low dry-matter intake (Van Soest 1994). Biomass yield was positively correlated with ADF ($r = 0.42$, $P < 0.0001$) and NDF ($r = 0.57$, $P < 0.0001$) in our study, probably because as plants grew, their leaf-to-stem ratio typically decreased as more structural tissue was produced to support plant growth. This was similar to that observed by Sheaffer et al. (2000). A negative correlation was reported between NDF and leaf fraction in Illinois bundleflower in Minnesota (DeHaan et al. 2003). In general, NDF was lower in basalt milkvetch (mean = 420 $g \cdot kg^{-1}$) than that for 15 native legumes from the north-central United States (mean = 571 $g \cdot kg^{-1}$ DM; McGraw et al. 2004). Values of ADF for the 15 native legumes (mean = 416 $g \cdot kg^{-1}$) were higher than those for basalt milkvetch (mean = 348 $g \cdot kg^{-1}$). Consequently, our results indicated that basalt milkvetch generally produces good quality forage.

Large plants of basalt milkvetch generally produced greater seed yields than small plants, as indicated by high positive correlations between biomass yield and seed yield ($r = 0.75$, $P < 0.0001$ in 2005, $r = 0.71$, $P < 0.0001$ in 2006). Larger plants typically have more resources to produce greater seed yields than smaller plants.

Seed mass varied significantly among basalt milkvetch accessions. Similar results were found with seed mass among ecotypes of sweetvetch (*Hedysarum boreale* Nutt.; Johnson et al. 1989). Within a species, heavier seeds typically produce seedlings with greater seedling vigor (Westoby et al. 2002) that can emerge from deeper soil depths (Johnson 1985) and are more likely to successfully establish under harsh environmental conditions (Asay and Johnson 1983; Milberg et al. 1998; Jakobsson and Eriksson 2000). As a result, accessions with heavy seeds may be of interest for plant improvement programs. In the present study, seed mass of basalt milkvetch was positively correlated with biomass in 2005 ($r = 0.49$, $P < 0.0001$) and 2006 ($r = 0.60$, $P < 0.0001$), similar to results for *Viola grypoceras* (Sakai and Sakai 1996). This was probably due to larger plants exploiting more water and nutrient resources, which allowed the production of bigger seeds. We also observed a tendency for plants from higher elevations to produce heavier seeds, which was reflected in the significant positive correlation between PC2 and elevation. Furthermore, a significant negative correlation between PC2 and mean annual minimum temperature suggests that temperature may play a role in this relationship.

Seed predation in basalt milkvetch can be as high as 80%, as documented in a study conducted in Oregon (Youtie and Miller 1986), which can severely limit species recruitment (Louda et al. 1990). Our study at Millville showed that basalt milkvetch treated with imadicloprid produced greater seed amounts than nontreated plants in 2006, but not in 2005 (Tables 4, 6). This may be due to the 72% lower seed yields observed in 2005 (compared to 2006). A nonsignificant insecticide treatment by accession interaction in our study indicated that insect predation was independent of accession. As a result, imadicloprid may be useful in enhancing seed production of basalt milkvetch.

A significant correlation between geographic distance and morphometric distance ($r = 0.34$, $P = 0.002$) indicated, as expected, that basalt milkvetch accessions collected in close proximity were more similar morphologically than more distant accessions. This relatively low correlation may result from basalt milkvetch being an out-crossing species with relatively low genetic diversity among accessions (Bussel 1999), as has been reported for another cross-pollinated species, *Adansonia digitata* (Assogbadjo et al. 2006).

We observed a high correlation between elevation and PC1 ($r = -0.71$, $P < 0.0001$). A positive correlation between elevation and longitude ($r = 0.62$, $P < 0.0001$) reflected that our eastern collection sites were from higher elevations than our western sites. Also, a negative correlation between elevation and latitude ($r = -0.84$, $P < 0.0001$) indicated that our southern collection sites were from higher elevations than our northern sites. Therefore, the observed negative correlation between elevation and PC1 may result from the high-elevation accessions being from Nevada, which typically has more severe environmental conditions than lower elevation sites in other states.

We observed in our field studies that basalt milkvetch exhibited the ability to develop new shoots from lateral roots, which is not typical of most *Astragalus* species (Barneby 1964). Creeping rootedness has been reported for alfalfa (*Medicago sativa* L.) and is believed to increase sward persistence under grazing (Pecetti and Piano 2002; Pecetti et al. 2004). Roots of creeping-rooted alfalfa accessions exhibit latent stem apices (Pecetti and Piano 2002). The ability to develop new shoots from lateral roots was observed in several basalt milkvetch accessions. We also observed that when the main crown died, plant shoots were sometimes able to regenerate by developing new crowns below the original crown. This regeneration capability may warrant further investigation.

MANAGEMENT IMPLICATIONS

Changes in rangeland botanical composition due to anthropogenic activities have increased fire frequency in the Great Basin (Whisenant 1990) and threatens the region's ecological and economic sustainability (Pellant et al. 2004). Establishing perennial vegetation on degraded rangelands can restore ecosystem function (Roundy et al. 1995), making rangeland ecosystem services more sustainable. Arid and semiarid rangelands are usually nitrogen limited, and only a few North American legumes are available for restoring rangelands in the Intermountain Region of the western United States (Johnson et al. 1989; Pyke et al. 2003). Basalt milkvetch is one legume that is widely distributed in western North America (Isely 1998), which lessens the concern that it could unnaturally hybridize with threatened *Astragalus* species (Steve Caicco, personal communication, May 2008). Thus, basalt milkvetch may be a particularly desirable species for revegetating rangelands in the western United States. Further, this species is not toxic to livestock and wildlife, exhibits a flush of growth after fire, possesses high forage quality, and may be competitive with *Bromus tectorum* L. Results from our studies provide important baseline data for identifying basalt milkvetch accessions that have potential for revegetation and restoration of degraded rangelands in areas ecologically similar to northern Utah.

ACKNOWLEDGMENTS

We would like to thank Drs Ron Ryel, Charles Romesburg, and Steve Larson for their valuable contributions to this research.

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